

Inferring the origin and dietary history of beef from C, N and S stable isotope ratio analysis

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Abstract

There is a pressing need for scientific methods that provide independent proof of the authenticity of animal produce for human consumption. Results of two feasibility studies suggest that the analysis of natural stable isotope compositions of carbon, nitrogen and sulphur is one potential tool for the verification of the geographical origin and feeding history of beef cattle. Beef reared in the USA (23 samples) and Brazil (10 samples) was isotopically different from northern European beef (35 samples), mainly because of contrasting proportion of plants with C₃ and C₄ photosynthetic pathways in the cattle diets. Combined C, N and S stable isotope ratio analysis also separated organically (15 samples) and conventionally (17 samples) produced Irish beef, even though underlying mechanisms are not yet fully understood.

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1. Introduction

Consumers demand reliable information about the food they buy. In particular, guarantees concerning the authenticity of meats are deemed fundamental to the assurance of food safety, quality and animal welfare (Verbeke & Viaene, 1999). About 10% of meat produced globally for human consumption is traded internationally (Barcos, 2001). Yet, existing livestock traceability schemes, based on tags, tattoos, life-numbers and animal passports, depend ultimately on a paper trail. There

is an urgent need for scientific, independent technologies for meat authentication to reassure consumers, protect regional designations and ensure fair competition (Ilbery, Kneafsey, & Bamford, 2000).

High-precision abundance measurements of naturally present stable isotope pairs of light elements have been used forensically to assign biological materials, such as cocaine (Ehleringer, Cooper, Lott, & Cook, 1999) and ivory (Vogel, Eglinton, & Auret, 1990) to restricted regions. The same technique is now well-established for the determination of the regional origin of honeys, juices, spirits, wines, oils and several other plant-derived foods (Kelly, 2003; Krueger, 1998; Rossmann, 2001). This is possible because plants, and non-migratory animals feeding on them, have potentially region-specific

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isotopic compositions determined by climatic and environmental conditions. However, the isotopic authentication of milk (Kornexl, Werner, Rossmann, & Schmidt, 1997) and meat is more complex because livestock can consume foodstuffs of various origins and can also be raised on several different farms during their lifetime. Further, extensive research on wildlife species (Kelly, 2000) suggests that most biological and physiological factors influencing the isotopic composition of animal tissues are still poorly understood.

Current research on light element isotopic beef authentication broadly pursues two main approaches. H and O isotope ratio analysis is applied to ascertain regional origins linked to regional climatic conditions (Boner & Förstel, 2004; Hegerding, Seidler, Danneel, Gessler, & Nowak, 2002; Renou et al., 2004). C and N isotope ratio analysis is mainly used to detect dietary components, such as maize or concentrates (Boner & Förstel, 2004; Gebbing, Schellberg, & Kühbauch, 2004; Quilter, 2002), but S has so far not been used. Results to date suggest that both avenues of research are promising.

The objective of the two feasibility studies reported here was to explore the usefulness of the C, N and S stable isotope composition as a potential marker of the international geographical origin and feeding history (conventional versus organic) of beef cattle.

2. Materials and methods

2.1. International samples

In the first, international study, samples of Belgian ($n = 2$), Dutch ($n = 3$), French ($n = 2$), German ($n = 5$), Italian ($n = 1$) and Brazilian ($n = 10$) beef were obtained from two licensed Irish meat importers. These meat samples (about 200 g each) had no background information other than country of origin, but were stated to be from different animals. Spanish samples ($n = 5$) were sourced through the Universidad Complutense de Madrid from a local commercial supplier. US samples (two lots, $n = 11$ and 12) came from research herds (University of Nebraska) fed typical diets high in maize for 85 and 180 d prior to slaughter. Conventional Irish samples (see the following section) were also included in this study.

2.2. Conventional vs. organic Irish samples

In the second study, samples of striploin or round steak from conventional ($n = 17$) and organic ($n = 15$) Irish beef were sourced at weekly intervals between February and April 2002 from supermarkets and certified organic butchers in the Dublin area. Animal identification data were obtained, including ear-tag or carcass

Table 1
Number of samples per county

County	Conventional ($n = 17$)	Organic ($n = 15$)
Carlow	1	–
Cork	5	–
Limerick	1	1
Louth	3	–
Meath	–	1
Tipperary	4	2
Waterford	1	–
Westmeath	1	5
Wexford	1	–
Wicklow	–	3
Uncertain	–	3 ^a

^a Meat cuts were packed in batches with more than one county of origin. Two of these samples came from either Meath or Tipperary, the third from four possible counties.

numbers, as well as code and address of the farmer who supplied the finished beef animal. Organic farms were accredited by the Irish Organic Farmers' and Growers' Association or the Organic Trust. All but 3 organic samples were traceable to the farm and thus county of origin (Table 1). The county origin of conventional and organic samples was reasonably similar and representative of beef-producing regions (Table 1). There were no isotopic patterns relating to county of origin (data not shown).

2.3. Sample processing and analysis

Upon collection, samples were vacuum-packed and stored at $-20\text{ }^{\circ}\text{C}$ prior to processing. Whole muscle subsamples (about 2 g fresh weight) were freeze-dried for 24 h and pulverized in a ball mill. Lipids were extracted from 100 mg muscle powder using the Radin method (Radin, 1981). Natural abundance stable-isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$) and sulphur ($^{34}\text{S}/^{32}\text{S}$) were measured on the de-fatted muscle residue by continuous flow isotope ratio mass spectrometry (Scrimgeour & Robinson, 2004) using a Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser (PDZ Europa Ltd., Northwich, UK). C and N were analysed in dual isotope mode, in duplicate; samples were 0.9–1.1 mg and precision (1 SD, $n = 3$, de-fatted bovine muscle) was 0.1‰ for both N and C. S was analysed on samples weighing 7.5–8.5 mg and 20% of all samples were analysed in duplicate; precision (1 SD, $n = 8$, bovine liver) for S was 0.14‰. Isotope ratios are expressed in delta (δ) notation in parts per thousand (‰).

Data were analysed by single factor Multivariate Analysis of Variance (MANOVA) based on Pillai's test statistic, using Minitab R13.20 (Minitab Inc., State College PA, USA). Univariate tests of normality (Kolmogorov–Smirnov) and equality of variances (Levene) suggested that all variables in both datasets met these two assumptions ($P > 0.10$) apart from $\delta^{13}\text{C}$. No

effective data transformations were found for $\delta^{13}\text{C}$. However, Pillai's statistic is generally considered to be robust in such situation (Quinn & Keough, 2002). In the text, data are reported as means \pm 95% confidence interval.

3. Results and discussion

3.1. International study

European beef (including conventional Irish) was significantly different from American beef (MANOVA, $F_{2,65} = 327.3$, $P < 0.001$) based on C and N isotopic compositions. The observed large difference in $\delta^{13}\text{C}$ between European and American beef (Fig. 1) can only be explained by contrasting proportions of plants with C_3 and C_4 photosynthetic pathways (Kelly, 2000; Minson, Ludlow, & Troughton, 1975) in the cattle diets. Mean $\delta^{13}\text{C}$ values of terrestrial C_3 and C_4 plants are -27‰ (range -35‰ to -21‰) and -13‰ (range -14‰ to -10‰), respectively (Kelly, 2000). Mean $\delta^{13}\text{C}$ values for conventional Irish ($-24.5\text{‰} \pm 0.7\text{‰}$) and other European ($-21.6\text{‰} \pm 1.0\text{‰}$) samples suggest a predominance of C_3 dietary ingredients derived from temperate, C_3 plants. By contrast, considerably less negative $\delta^{13}\text{C}$ values for US ($-12.3\text{‰} \pm 0.1\text{‰}$) and Brazilian ($-10.0\text{‰} \pm 0.6\text{‰}$) beef reflect the almost exclusive use of C_4 foodstuffs, likely maize or (sub)tropical C_4 pasture grasses (Minson et al., 1975).

These results identify $\delta^{13}\text{C}$ as a single marker that distinguishes American from European beef. This probably holds true for northern Europe, including Ireland and Britain, where pastoral beef production systems predominate and use of the only C_4 crop, maize, is marginal. Published independent $\delta^{13}\text{C}$ measurements of muscle and hair from British beef support this view

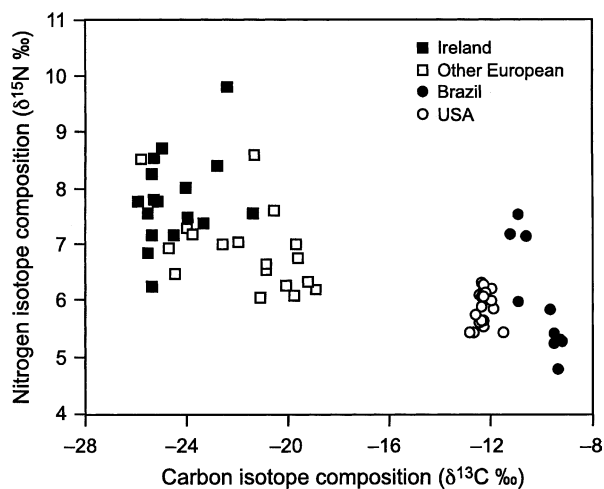


Fig. 1. C and N stable isotope ratios in de-fatted bovine muscle tissue (International study).

(Bol & Pflieger, 2002; Morrison, Dodson, Slater, & Preston, 2000). However, the present study does not reflect the fact that beef rearing systems with high maize usage exist in central and southern Europe (Bonner & Förstel, 2004; Gebbing et al., 2004). For example, beef muscle $\delta^{13}\text{C}$ values ranged from -24‰ to -13‰ in a local survey of 23 farms in southern Germany, while controlled grass-fed beef had a mean $\delta^{13}\text{C}$ of -27‰ (Gebbing et al., 2004).

Interestingly, Irish conventional beef was also significantly different from other European beef (MANOVA, $F_{2,32} = 14.8$, $P < 0.001$) based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. This result suggests that isotopic origin authentication of beef may work on smaller geographic scales.

3.2. Conventional vs. organic Irish beef

Organically produced beef, which enjoys growing demand and attracts premium prices (Verbeke & Viaene,

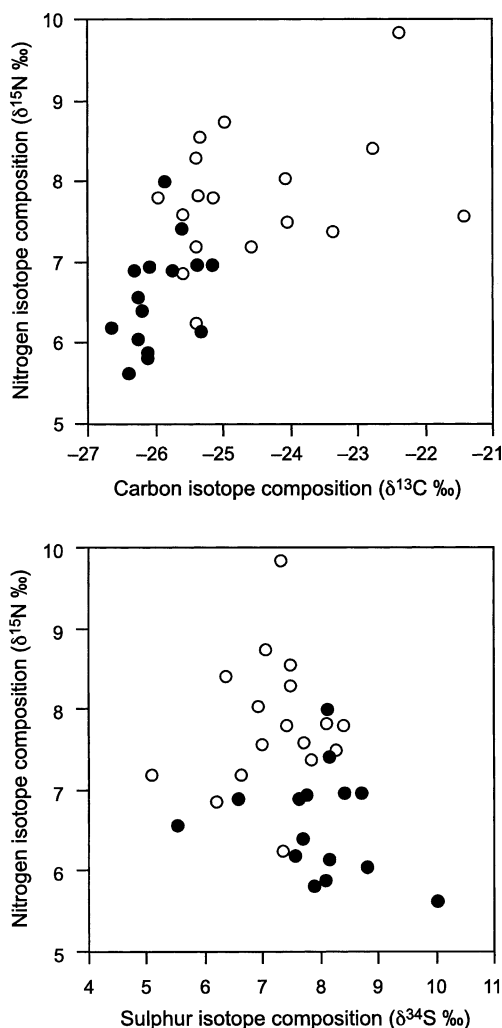


Fig. 2. C, N and S stable isotope ratios in de-fatted muscle tissue from beef cattle raised in Ireland in conventional (hollow circles) or organic (solid circles) production systems.

1999), cannot be authenticated independently at present. In the present study, the combined isotopic composition of C, N and S distinguished between conventional and organic Irish beef (Fig. 2, see also Figure 3 *Electronic supplementary material*). The two groups were significantly different (MANOVA, $F_{3,28} = 10.3$, $P < 0.001$).

Conventional Irish beef had a less negative and somewhat more variable $\delta^{13}\text{C}$ value ($-24.5\text{‰} \pm 0.7\text{‰}$) than organic beef ($-26.0\text{‰} \pm 0.2\text{‰}$). These data suggest that more concentrated foodstuffs are fed in conventional than in organic production which relies more on grass which has more negative $\delta^{13}\text{C}$ values than concentrates (Schmidt et al., 2002). This pattern has also been reported in an isotopic survey of milk in Germany (Kornel et al., 1997). Possibly, some C_4 foodstuffs (maize, sugar cane molasses) were fed to conventional cattle in the few cases where muscle $\delta^{13}\text{C}$ was higher than -24‰ (Fig. 2). Boner and Förstel (2004) also reported a narrow range of $\delta^{13}\text{C}$ values in a survey of organic German beef samples (85% of the 223 samples were between -27‰ and -25‰) and concluded that $\delta^{13}\text{C}$ is a strong candidate marker for authentication of organic beef production.

Conventional beef had higher $\delta^{15}\text{N}$ values ($7.8\text{‰} \pm 0.4\text{‰}$) than organic ($6.6\text{‰} \pm 0.4\text{‰}$) beef (Fig. 2). It can be hypothesized that, in these predominantly pastoral production systems, this result reflects a cumulative, plant–soil-system ^{15}N enrichment under conventional, more intensive management which is associated with external N inputs and hence higher N (preferentially ^{14}N) losses from such ‘open’ systems (Hebert & Wassenaar, 2001). A similar dynamic might explain $\delta^{15}\text{N}$ patterns in the international dataset (Fig. 1). However, alternative explanations (e.g. higher legume content in organic systems) cannot be ruled out.

There was also a small enrichment in $\delta^{34}\text{S}$ in organic ($7.9\text{‰} \pm 0.6\text{‰}$) compared to conventional ($7.2\text{‰} \pm 0.4\text{‰}$) Irish beef (Fig. 2). The reasons for this enrichment are not clear and it does not conform with documented long-term $\delta^{34}\text{S}$ changes in English soils receiving either organic or mineral fertilizers (Knights, Zhao, Spiro, & McGrath, 2000). Perhaps it reflects the use of seaweed, which is enriched in ^{34}S compared to terrestrial sources, as a feed supplement or fertiliser on organic farms.

3.3. Conclusions

It is commonly said that all of Ireland’s beef production is ‘organic’ because it is pasture based and often extensive. Initial results suggest that isotope ratio analysis can detect differences between very similar agricultural production systems, even though underlying mechanisms are not yet fully understood. The feasibility of inferring geographical origins from isotopic composi-

tions has also been established. C, N and S stable isotope ratio analysis has potential as one of the tools for a chemometric approach to meat authentication (Heaton, Kelly, & Hoogewerff, 2003) incorporating analysis of heavy element isotopes, trace elements and fatty acid composition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.foodchem.2004.08.036](https://doi.org/10.1016/j.foodchem.2004.08.036).

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